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8 Inflammation in Parkinson's disease: Therapeutic implications

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Abstract

Parkinson's disease (PD) is known to be a chronic and progressive neurodegenerative disease caused by a selective degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc). A large body of experimental evidence indicates that the factors involved in the pathogenesis of this disease are several, occurring inside and outside the DAergic neuron. Recently, the role of the neuron-glia interaction and the inflammatory process, in particular, has been the object of intense study by the research community.

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It seems to represent a new therapeutic approach opportunity for this neurological disorder. Indeed, it has been demonstrated that the cyclooxygenase type 2 (COX-2) is up-regulated in SNc DAergic neurons in both PD patients and animal models of PD and, furthermore, non-steroidal anti-inflammatory drugs (NSAIDs) pre-treatment protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6 hydroxydopamine (6-OHDA)-induced nigro-striatal dopamine degeneration. Moreover, recent epidemiological studies have revealed that the risk of developing PD is reduced in humans who make therapeutical use of NSAIDs. Consequently, it is hypothesized that they might delay or prevent the onset of PD. However, whether or not these common drugs may also be of benefit to those individuals who already have Parkinson's disease has not as yet been shown.

In this paper, evidence relating to the protective effects of aspirin or other NSAIDs on DAergic neurons in animal models of Parkinson's disease will be discussed. In addition, the pharmacological mechanisms by which these molecules can exert their neuroprotective effects will be reviewed. Finally, epidemiological data exploring the effectiveness of NSAIDs in the prevention of PD and their possible use as adjuvants in the therapy of this neurodegenerative disease will also be examined.

Introduction

Parkinson's disease (PD) is the most prevalent neurological disorder of the basal ganglia, and it is characterized by a progressive loss of dopaminergic (DAergic) neurons in the caudate nucleus, putamen and substantia nigra (SN) [1,2]. The loss of DAergic neurons in the substantia nigra pars compacta (SNc) is the principal feature of PD [3] and results in cardinal motor symptoms such as tremor at rest, bradykinesia, muscular rigidity, stooped posture and instability [4]. Hitherto, despite the recent progress in understanding the etiopathogenesis of PD, the modalities whereby the neurodegenerative process begins and progresses are still unclear. Furthermore, the situation is complicated by the large number of factors that seem to be involved in the onset of this disease, such as aging, genetic vulnerability, exogenous or endogenous toxins, hydroxyl radicals ($^{\cdot}OH$) production, neuronal metabolic disturbances and inflammation [4-8]. Thus, the cumulative neuronal insults attributable to these metabolic stress factors may promote premature SNc DAergic degeneration through the activation of apoptotic programs [9-11]. However, the specifics and sequential neuroapoptotic events associated with premature, progressive SNc neuronal atrophy remain undefined.

Thus far, among the various accepted experimental models of PD, neurotoxins still represent the most popular tools to produce selective death of

DAergic neuron both *in vitro* and *in vivo* systems. Even though recent genetic discoveries have lead to a number of different genetic models of PD, none of these shows the typical degeneration of DAergic neurons [12,13]. Among the neurotoxins, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a product of synthetic meperidine derivative, and 6-hydroxydopamine (6-OHDA), hydroxylated dopamine derivatives are the most used for inducing parkinsonian features in cells and animal species. MPTP is metabolized to the 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase-B (MAO-B) [14]. This highly toxic metabolite is selectively taken up into dopaminergic neurons, via the DA transporter [15], where it provokes an intracellular accumulation of Ca^{2+}, thus interfering with the function of nerve terminals in the striatum [16] and inhibits complex 1 (NADH-ubiquinone oxidoreductase) of the respiratory chain causing progressive cell death [17]. On the other hand, the neurotoxic effects of 6-OHDA are mediated by the generation of $^{\cdot}OH$, pro-inflammatory mediators or pro-apoptotic agents [18-20]. The result of the administration of each neurotoxin, although by different mechanisms, is DA depletion in the nigro-striatal pathway of laboratory animals and molecular alterations comparable to those seen in PD's patients [21]. Recently, it has been shown that 6-OHDA, MPTP as well the bacterial lipopolysaccharide (LPS) induce the death of DA cells activating an immune response [22-24]. These animal models have been crucial in the study of PD and have allowed the formulation of different hypotheses about its etiopathogenesis, and recently, they have been utilized to determine the role of inflammation in DA neuronal death. Moreover, toxin-based models have been useful in developing neuroprotective and neurorestorative strategies and in testing new drugs for the treatment of this disorder. In this review, experimental data regarding the role of neuroinflammation in the aetiology of PD, the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and the possibility for their use as a new therapeutic approach for this neurodegenerative disease will be reviewed.

Inflammation in Parkinson's disease

Thus, decades of research on the aetiology of Parkinson's disease has resulted in much information, but little has been gained in establishing the events causing the initiation and progression of the disease. Recently, the involvement of neuroinflammation and microglial activation in the pathogenesis of PD has been emphasized [25,26]. Results of neurotoxin models of PD, corroborating findings obtained in transgenic animal models and epidemiological studies, strongly support the hypothesis that this neurodegenerative disease is not purely neuronal, as it has been previously considered [27,28]. Thus, DAergic neuronal degeneration is the likely result of multiple pathogenic factors occurring both within and outside the cell. The

cross-talk between neurons and glia is becoming more and more important for the understanding of brain pathophysiology. This new finding, unfortunately, does not allow us to diagnose the disease any earlier because the neuroinflammatory process is silent and unnoticed due to the absence of pain fibres in the brain, but it at least gives a glint of hope for new potential therapeutic targets for the slowing of neuronal degeneration.

Neuroinflammation is not a distinctive characteristic of PD but it has been clearly revealed in a broad spectrum of neurodegenerative diseases that share with it a common pathological process, such as Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) [26,29]. The scenario is still obscure, but inflammation in PD is not any longer considered a non-specific consequence of neuronal degeneration as it was originally thought to be. Indeed, neuroinflammation may aggravate the course of the disease and, as it has recently been suggested, may be a primary factor in some cases of PD [8,26,27,30]. Indeed, postmortem examinations have shown that neuronal degeneration in PD is associated with massive gliosis due to a subset of activated glial cells, the microglia [31-33], evidence that has been confirmed in MPTP-induced parkisonism in monkeys [34] and humans [35]. Interestingly, the SN, usually prone to the deleterious effects of oxidant stress, containing DA neurons high in iron and low in glutathione [8,36], is also one of the brain regions more sensitive to inflammation. Indeed, healthy SN exhibits the highest concentration of microglia in the brain especially in the ventral tier of the pars compacta [37,38]. Normally, very few microglial cells are detected in the vicinity of DAergic neurons, and when present, they appear to be resting with fine, long processes. Neuronal damage, aggregated proteins with abnormal conformations present in Lewy bodies and other unknown factors increase the number and change the shape of glial cells, to such an extent that they can be found in proximity to DA cells with short cellular processes [39]. Activated microglia are recruited to the SNc from various structures and finally stuck to DA neurons. It has been shown that glial cells once activated become phagocytes that ingest degenerating DA neurons piece-by-piece. This occurs early in neuronal degeneration, starting at the extending fibres, such as the neurite which extend into the SN reticulata [40]. Hence, activated glial cells release detrimental compounds such as, interleukin (IL)-1β, IL-6, tumor necrosis factor- α (TNF-α) and interferon γ (IFN- γ), which may act by stimulating inducible nitric oxide synthase (iNOS), or which may exert a more direct deleterious effect on DAergic neurons by activating receptors that contain intracytoplasmic death domains involved in apoptosis [24,33,41-48]. Microglia can also induce neuritic beading [49] or synaptic stripping along dendrites [50]

leading to synaptic disconnection and loss of trophic support and cell death [51,52]. Animal studies using MPTP have shown that the immune reaction might evolve, ultimately leading to the infiltration of lymphocytic $CD4^+$ and $CD8^+$ T cells into the injured SN and striatum, given that glial cells are potent activators in lymphocyte invasion. Moreover, activated lymphocytes present in the SN could start an immune-mediated inflammation [47,48].

Nevertheless, such activation of microglia is not only disadvantageous to neurons. Indeed, some investigations indicate that activated microglial cells and macrophages tend to synthesise and produce neurotrophic factors (brain-derived neurotrophic factor, BDNF and glia-derived neurotrophic factor, GDNF) through certain compensatory mechanisms following neuronal injury and induce sprouting surrounding the wound in the striatal DA terminals [53,54]. Moreover, activated glia play a role in gradually removing the dead DA neurons as a defence mechanism, although some healthy DA neurons might be also phagocytosed during the process [9,55]. Therefore, inflammation has been rightly defined as a double-edged sword. It normally starts as a defence reaction but, for the failure of its control mechanism, can lead to an uncontrolled and continuous extremely damaging immune response. A brief pathogenic insult, furthermore, can induce an ongoing inflammatory response and the toxic substances released by the glial cells may be involved in the propagation and perpetuation of neuronal degeneration. This theory is plausible, corroborated by the evidence that several years after the exposure to MPTP, increased levels of factors such as, interleukin (IL)-1β, IL-6 and tumor necrosis factor- α (TNF-α) have been found in the basal ganglia and cerebral spinal fluid (CSF) of patients with toxin-induced PD [25].

A prominent factor in neuroinflammatory reactions in PD seems to be the activation of the complement system [56-58] a major mediator of immune/inflammation reactions. Indeed, increased mRNA levels of complement components have been found in affected brain regions [28]. The presence of complement components, including all constituents of the membrane attack complex (MAC), has been shown intracellularly on Lewy bodies and on oligodendroglia in the SN of PD patients [59-61]. Accumulation of Lewy bodies can apparently cause the activation of complement, the initiation of reactive changes in microglia, and the release of potentially neurotoxic products such as the MAC, $^{\cdot}OH$, and excess glutamate (GLU) [62].

So far, among the plethora of toxic factors released by the reactive glia it is not clear which one of them is responsible for the DAergic neuronal death. Reactive oxygen species (ROS), $^{\cdot}OH$, NO and its peroxinitrite ($ONOO^-$), are the likely candidates. From this evidence it appears clear that inflammatory process and oxidative stress derived from DA metabolism, constitute a vicious cycle that lead to the final demise of nigral DA cells (Fig. 1) [27].

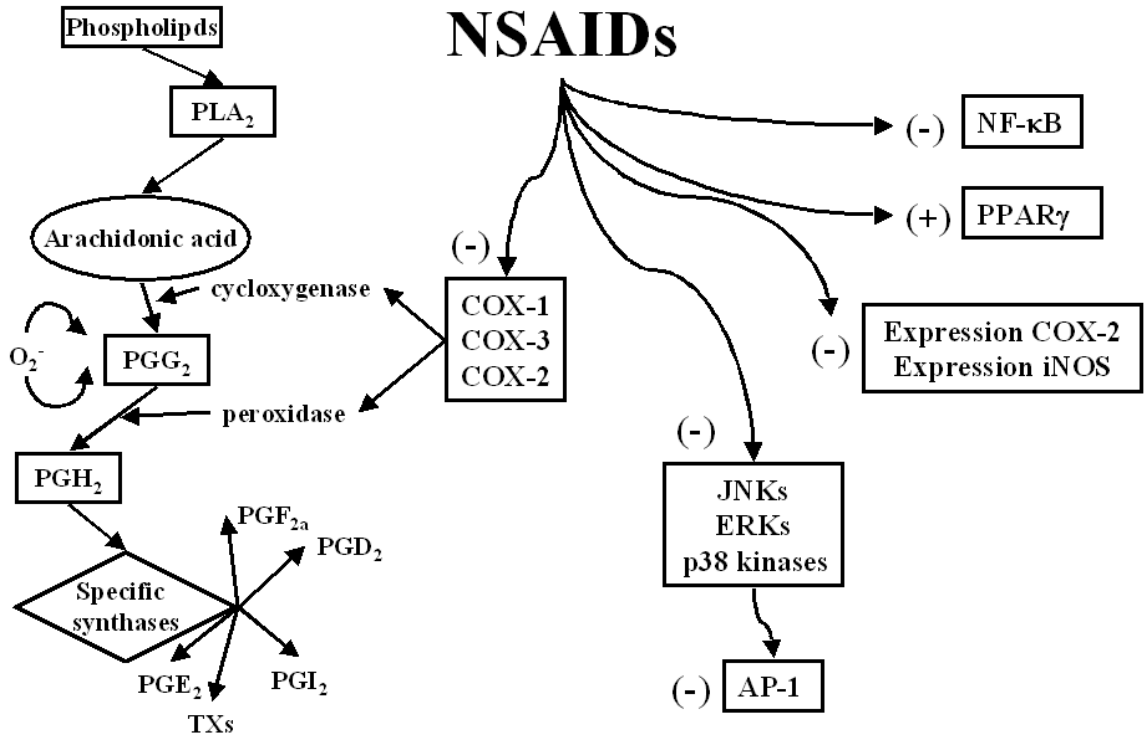

Figure 1. Classical versus non-classical effects of NSAIDs. Abbreviations: COX cyclooxygenase; PLA_2, phospholipase A_2; PGG_2, Prostaglandin G_2; PGH_2, Prostaglandin H_2; Prostaglandin F_{2alha}, $PGF_{2\alpha}$; Prostaglandin D_2, PGD_2; Prostaglandin I_2, PGI_2; Prostaglandin E_2, PGE_2; Thromboxanes, TXs; Nuclear Factor kappa B, NF-κB; Peroxisome proliferator-activated receptor gamma, PPARγ; Inducible nitric oxide synthase, iNOS; c-Jun N-terminal kinases, JNKs ; Extracellular signal-regulated kinases, ERKs; P38 mitogen-activated protein kinases, p38 kinases; factor activator protein 1, AP-1.

Furthermore, experimental evidence has also shown that inflammatory loss of DA nigro-striatal neurons might be mediated by apoptosis [63-67]. Indeed, inflammation induced by intranigral injection of LPS could be mediated, at least in part, by the mitogen-activated protein kinase p38 (MAPK p38) signal pathway leading to activation of inducible nitric oxide synthase (iNOS) and cysteine protease caspase-11 [64]. According to this evidence, it has been recently shown that LPS-induced inflammation causes apoptosis in the SNc due to increased pro-inflammatory cytokine levels of mRNA for TNF-α, IL-1α, IL-1β and IL-6, and the apoptosis-related genes Fas and Bax and caspase-3 immunoreactivity [63]. These data have been confirmed also in a MPTP mouse model, neurotoxic effect seems to be mediated via activation of the caspase-11 cascade and inflammatory cascade, as well as the mitochondrial apoptotic cascade [65].

Furthermore, the link between inflammation and apoptotic signalling cascade could follow other pathways. Thus, in a chronic MPTP model of PD, activation of the nuclear transcription factor NF-κB, that is well-known for its role in preventing apoptotic cell death, has been revealed [68], this in turn, promotes the synthesis of

cyclooxygenase types 2 (COX-2) [69]. COX-2 induction, increases inflammatory response with ROS formation by the arachidonic acid (AA) cascade, thus triggering a vicious circle (Fig. 1). The release of AA also inhibits GLU uptake contributing to the neurodegenerative processes seen in PD [70]. In addition, COX-2 could also be induced by pro-inflammatory cytokines such as TNF α via the c-Jun N-terminal kinase (JNK) pathway [33,71,72].

Old and new mechanisms of action for NSAIDs

The above discussion makes it plausible that drugs with the capacity to rescue DA neurons from microglia toxicity and inflammatory processes, may result in an amelioration of parkisonian symptoms by delaying the onset and slowing the progression of the disease [30,73,74]. Several agents have been shown to inhibit microglial or monocytic cell neurotoxicity [73,75]. Among them much attention has been devoted to NSAIDs since it has been shown by experimental and clinical observation that they may represent a possible new therapeutic approach for treating PD.

NSAIDs are an heterogeneous group of compounds which share many pharmacological properties (and side effects) and are the main drugs used as analgesics and antipyretics to reduce the untoward consequences of inflammation. NSAIDs together with steroidal anti-inflammatory drugs (SAIDs) are capable of halting eicosanoids synthesis and suspending inflammatory process progression. SAIDs, which include both cortisone and its derivatives, inhibit phospholipase A_2 (PLA_2) blocking both the production of LTs and PGs via the discontinuance of AA synthesis. Differently, NSAIDs only inhibit COX activity inducing a diminution of PGs levels, accompanied by a compensatory increase of LTs levels (Fig. 2).

The NSAIDs can be classified into three groups based on their COX inhibition ratios (affinity of inhibition for COX-1 or COX-2) chemical structures or inhibitory kinetics [76] (Table 1).

Recently, a new class of NSAIDs has been synthesized named nitric oxide-donating nonsteroidal anti-inflammatory drugs (NO-NSAIDs), consisting of a traditional NSAID to which a NO releasing moiety is covalently attached, that may have an important role in colon cancer prevention and/or treatment [77]. The main pharmacological action of these compounds is on the metabolism of AA inhibiting the enzymes possessing COX activity (Fig. 2) [78]. Whenever an inflammatory process occurs, there is a consequent activation of specific enzymes in the cell wall. Among them, one of the first activated is PLA_2 phospholipase A_2 (PLA_2) that deacylates fatty acids from the 2nd carbon atom of the triglyceride backbone of mambrane phospholipids, producing AAs and lyso-phospholipids [79-81]. AA is subjected to the action of two families of enzymes: lipoxygenases (LOX) and

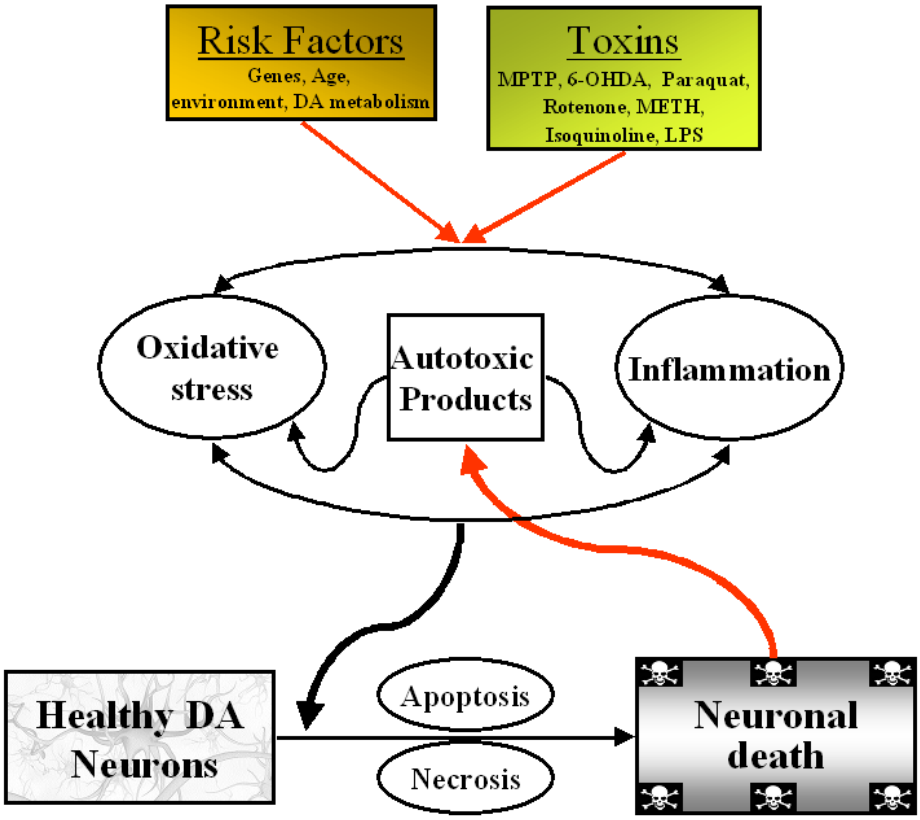

Figure 2. Interacting synergistic mechanisms involved in dopaminergic death in Parkinson's disease. The role of the positive feed back (vicious) circle between neurononal death, neuroinflammation and/or oxidative stress is depicted. Abbreviations: Dopamine, DA; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP; 6 hydroxydopamine, 6-OHDA; Methamphetamine, METH; lipopolysaccharide, LPS.

COX. These enzymes are able to insert oxygen into the molecule of AA in a specific way, producing five prostanoids: PGE_2, PGF_2, PGD_2, PGI_2 (prostacyclin), and thromboxane A_2 (TxA_2) through the intermediate PGH_2. These prostanoids bind to specific G-protein-coupled receptors designated EP (for E-prostanoid), FP, DP, IP, and TP receptors, respectively [82,83]. LTs are commonly known as vasoconstrictor and bronchospastic agents whereas PGs play a pivotal role in all the biochemical mechanisms inducing pain, hyperpyrexia and classical signs of inflammation, cytoprotective and citotoxicity processes. Bergstrom and colleagues [84] first described PGs in the brain more than 40 years ago. Since then, numerous studies have shown that PGs are formed in certain regions of the brain and in the spinal cord as a response to a variety of stimuli and in 1976 the enzyme which is key in the synthesis of PGs from AA, COX, was purified [85]. Subsequent to the cloning of the COX-1 gene, Dan Simmons and colleagues identified a second gene with COX activity (COX-2) [86]. Recently, a third variant of COX has been described, initially called COX-3

Table 1. Biological, pharmacokinetic and chemical subdivision of NSAIDs.

COX-2/COX-1 RATIO	INHIBITION KINETICS	CHEMICAL STRUCTURE
Nonselective COX inhibitors *(e.g.* ketorolac or piroxicam, with ratio ≈1);	simple, competitive *(e.g.* ibuprofen and Naproxen)	Carboxylic acids *(e.g.Aspirin and Ibuprofen)*
Selective COX-1 inhibitors *(e.g. Dexketoprofene and SC 560* with ratio < 0.01*)*	competitive, time-dependent, reversible *(e.g. Indomethacin and DuP 697)*	Pyrazoles *(e.g.Phenilbutazone and Kebuzone)*
Preferential COX-2 inhibitors *(e.g.* ibuprofen and indomethacin, with ratio 15-60)	competitive, time-dependent, irreversible *(e.g. Aspirin and Valeryl salicylate)*	Oxicams *(e.g. Piroxicam and Isoxicam)*
Selective COX-2 inhibitors *(e.g.* coxibs, selective COX-2, with ratio >1000)		Sulphonamides *(e.g. Valdecoxib and Celecoxib)*
		Methylsulphones *(e.g. Rofecoxib and Etoricoxib)*
		Arylacetic acid *(e.g. Lumiracoxib)*

[87,88], that might be the target of acetaminophen (paracetamol) [87-88]. COX-3 has been more appropriately renamed COX-1b being a splice variant of COX-1which has retained intron-1 during translation [88]. It has a completely different amino acid sequence than the known cyclooxygenases and it does not seem to show cyclooxygenase activity in mice [89] and rats [90], thus it may well be that COX-1b is not relevant to humans.

The properties of COX-1 are different to those of COX-2. It was originally thought that the function of constitutive COX-1 was involved in physiological phenomena, such as cytoprotection of the stomach, platelet aggregation, and kidney functions, whereas that of COX-2 was involved in various pathologies. However, recent studies suggest that the inducible isoform COX-2 also plays important role in development and homeostasis [91]. In the central nervous system (CNS), COX-2 plays an important role in membrane excitability, synaptic transmission and participates in memory consolidation during REM sleep [92,93].

COX-1 and COX-2 are widely and both constitutively expressed under normal physiological conditions in human organs [94], even though only COX-2 is dramatically up-regulated during inflammatory processes. Similarly to the other tissues, the two isoforms are distributed heterogeneously among the brain cells, COX-1 and COX-1b are detected in microglial cells, while COX-2 is found in neuronal and glial cells, astrocytes do not express significant COX levels [95,96]. Normally, COX-2 is expressed in low levels in nigral DA neurons, but it becomes up-regulated in both patients and experimental PD models [22-24,33,97-99].

COX-2 expression in neurons has been proposed to increase the vulnerability of neurons to GLU mediated excitotoxicity. In the CNS, COX-2 expression is increased in neurons following GLU receptor activation [100-102] and is thought to contribute to increased neuronal death. Genetic evidence also indicates that neuronal expression of COX-2 leads to excitotoxic cell death. Transgenic mice that overexpress neuronal COX-2 are more susceptible to excitotoxic cell death [103] and age associated neuronal loss [104]. This evidence has been confirmed in rat ischemic hippocampus, where COX-2 expression was substantially and significantly upregulated in vulnerable CA1 and not in resistant CA3 and dentate granule cells [105]. In contrast, COX-2 null (knockout) mice exhibit less neuronal death following ischemia, challenge with NMDA [106], and MPTP [22,98,99,107]. Pharmacological and genetic inhibition of COX-2 is capable of sheltering DA neuronal bodies in the SNc as well as the striatal TH-stained fibres against toxin effects, suggesting a protection of the entire nigrostriatal pathway [22]. Potential downstream effectors of COX-2 neurotoxicity on SNc DA neurons are PGE_2 and $^{\cdot}OH$ generated through peroxidase activity, levels of which have been found to be enhanced in experimental models and in PD post-mortem samples [22,33,97,108]. PGE_2 mediates COX-2 neurotoxicity essentially through the activation of EP_1/EP_3 receptors that disrupt Ca^{2+} homeostasis by incresing its cellular concentration thus causing excitotoxic neuronal death [102,109]. Conversely, the activation of the prostanoid EP_2/EP_4 G-protein-coupled receptors seems normally to be associated with neuroprotection [110,111]. PGE_2 is present in ventral midbrain neurons and derives primarily from COX-1 [33]. After a few days of neuronal insult, PGE_2 concentration almost doubles due to MPTP-induced COX-2 up-regulation, although more than half still depends on COX-1 activity [33]. Microglia-DAergic neurons interaction is necessary for MPP^+-induced COX-2 activation and PGE_2 production [23]. The toxin first induces reactive microgliosis and secretion of its proinflammatory factors, among them PGE_2. These will enhance COX-2 DA neuronal activity and lead to a second wave of neuronal damage, which in turn, could reinforce the microgliosis process. The strong correlations found between COX-2 and PGE_2 levels, microglial activation and dopaminergic neurodegeneration suggest that COX-2 may mediate microglial activation and may play a key role in amplifying the inflammatory response and other toxic effects in a vicious circle, which ultimately exacerbates dopaminergic neuronal loss (Fig. 1) [22,23,33,40].

Moreover, COX-2 activation might result in direct DAergic cell demise by producing the neurotoxic oxidant species DA-quinone [33,112], and by increasing DNA damage inducing the formation of etheno-DNA adducts that arise as a consequence of COX-2-mediated lipid peroxidation [113,114].

Aspirin (acetylsalicylic acid, ASA) is the most frequently used drug in the world to treat inflammation and pain. Aspirin is the progenitor of the NSAIDs family, it is known to preferentially inhibit COX-1 rather than COX-2 in an irreversible way, by acetylating the active site of these enzymes, producing salicylic acid (SA) [80,115]. Although many of ASA's and other NSAIDs' pharmacological actions are related to the ability to inhibit prostaglandin biosynthesis, some of their beneficial therapeutic effects are not completely understood. NSAIDs are able to inactivate the transcription factors NF-κB and factor activator protein 1 (AP-1) which is critical for the induction of neoplastic transformation and the induction of multiple genes involved in inflammation and infection [115-121]. Diverse noxious cellular stimuli free NF-κB from any endogenous inhibitor, permitting the translocation of free NF-κB from the cytoplasm to the nucleus. Consequently, NF-κB binds to DNA and activates a number of genes involved in the inflammatory and immune responses. Some of these gene products, such as TNF could exert cytotoxic effects by switching on apoptotic self-destruct programs [122,123]. ASA and COX-2 selective inhibitors exert antitumor effects partly through blocking AP-1 activation. AP-1, consisting of Jun/Fos dimers, is a downstream target of MAP kinase family members including extra-cellular signal regulated kinases (ERK-1 and -2; p42/p44 MAPK), Jun kinases (JNK), and p38 MAPK. NSAIDs suppress AP-1 activation through different mechanisms blocking the activation of ERK and JNK as well as P38 mitogen-activated protein kinase (p38 kinase) [118,124]. Furthermore, ASA and salicylate at therapeutic concentrations inhibit COX-2 protein expression pointing towards a possible (cell-specific) target of NSAIDs upstream to COX-2 enzyme activity through interference with the binding of CCAAT/enhancer binding protein beta (C/EBPbeta) to its cognate site on COX-2 promoter/enhancer. Expression of other genes, such as iNOS and interleukin-4, may be inhibited by ASA and salicylate through a C/EBP-dependent mechanism or inhibiting NF-κB activation [125,126].

Among the COX independent actions, it has been shown that NSAIDs in neuronal cells, might directly and dose-dependently scavenge ROS and reactive nitrogen species (RNS) blocking their detrimental effects [117,127]. Moreover, the agonistic activity shown at high concentration by some NSAIDs such as ibuprofen and indomethacin toward the peroxisome proliferator-activated receptor-γ (PPARγ) seems relevant to neuroprotection [128]. This receptor PPARγ is a ligand-activated inhibitory transcription factor that antagonizes the activity of NF-κB, AP-1, signal transducer and activator of transcription 1 (STAT-1) and nuclear factor of activated T cells (NFAT) [129,130]. Its cellular activation is associated with a reduction in the expression of several inflammatory genes [131] and the production of inflammatory cytokines (i.e., IL-1, IL-6, TNF) [130]. In vitro studies have

shown that the selective agonists pioglitazone, indomethacin and ibuprofen can activate PPARγ in microglia, reducing the Aβ-mediated secretion of inflammatory cytokines and neurotoxicity, decreasing the number of activated microglia and reactive astrocytes [132,133]. Drug treatment reduces the expression of the proinflammatory enzymes COX-2, iNOS and beta-secretase-1 (BACE1) mRNA and protein levels [133]. In addition, PPARγ depletion potentiates beta-secretase mRNA levels by increasing BACE1 gene promoter activity. Conversely, overexpression of PPARγ, as well as NSAIDs and PPARγ activators, reduced BACE1 gene promoter activity. These recent results suggest that PPARγ could be a repressor of BACE1 binding to a PPRE located in the BACE1 gene promoter. These effects may explain the overexpression of BACE1 in the brain under inflammatory conditions and emphasize the hypothesis that neuroinflammatory mechanisms significantly contribute to the pathogenesis of AD. This could be a potential mechanism by which NSAIDs have a protective effect against the development of AD [134].

Currently, selective COX-2 inhibitors are used more frequently than the other NSAIDs because they produce the same pharmacological effects as non selective COX inhibitors without the attendant COX-1 inhibition related toxic effects on stomach lining. Unfortunately, this class of drugs has recently been shown to increase the risk of cardiovascular events. As a result, some of these drugs have been withdrawn from the market and some clinical trials halted, among them a trial with celecoxib in AD [135].

Neuroprotective effects of NSAIDs in Parkinson's disease: Experimental evidence

Since the end of the 1980s, when McGeer and colleagues [31] in their seminal study reported a large number of reactive leucocyte antigent-DR (HLA-DR)-positive microglial cells in the SNc and striatum of patients with PD, several experimental investigations have provided further plausible evidence for the activation of a proinflammatory response in this disease. The importance of the subject, has engaged several groups in the emerging and promising theme of NSAIDs and neurodegeneration. As far as we are aware, nineteen studies have been carried out in which the effects of NSAIDs have been tested on animal (mouse and rat) models of PD and cell cultures (Table 2). This evidence supports the use of NSAIDs in reducing the pathological burden of the disease; ASA was the most tested drug (8 studies), followed by its metabolite SA (5 studies), only 1 studied the effect of COX-1 but 10 focused on the role of COX-2 using selective inhibitors for this isoform of the enzyme. The results of ten years of research will be reported chronologically.

The first piece of experimental evidence in the field was published by Grilli and co-workers a few years after the McGeer study [117]. These authors showed that ASA and its metabolite SA, at concentrations compatible with amounts in plasma during treatment of chronic inflammatory states, were protective against neurotoxicity elicited by GLU in primary cultures of rat cerebellar granule cells and hippocampal slices, whereas indomethacin was unable to prevent GLU-induced cell death. The common molecular target for

Table 2. Experimental studies with NSAIDs.

Experimental model	NSAIDs	Outcome
primary cultures of rat cerebellar granule cells and hippocampal slices [117]	ASA (1, 3 mM) SA (3,10 mM)indomethacin (1-20μM)	Protection ↓NF-κB Protection ↓NF-κB NO protection
MPTP mouse model of PD, MPTP (15 mg/kg, s.c.) [136].	ASA (100 mg/kg) Aspegic (200 mg/kg) SA (100 mg/kg) Paracetamol (100 mg/kg) Diclofenac (100 mg/kg) Ibuprofen (20 mg/kg) Indomethacin (100 mg/kg)	Protection ROS scavenging Protection ROS scavenging Protection ROS scavenging NO protection NO protection NO protection NO protection
MPTP mouse (C57BL/6) model of PD, MPTP 30 mg/kg or 40 mg/kg s.c. [140].	SA (50 mg/kg or 100 mg/kg i.p.)	Protection ROS scavenging
MPTP mouse model of PD, MPTP (30 mg/kg i.p. twice, 16 h apart) 138].	SA (25-100 mg/kg, i.p.)	Protection ROS scavenging ↓akinesia or catalepsy
Cultured primary rat embryonic neurons from mesencephalon GLU-toxicity [139].	ASA (1 mM) Paracetamol (1 mM) Ibuprofen (0.1 mM)	Protection ??Mechanism Protection ??Mechanism Protection ??Mechanism
MPTP mouse (C57BL/6) model of PD, MPTP (30 mg/kg s.c.) [33]	ASA (10, 50, 100 mg/kgi.p.). Meloxicam (2, 7.5,50 mg/kg i.p.)	Protection ↓COX-1/ COX-2 ↓akinesia or catalepsy Protection ↓COX-2 ↓akinesia or catalepsy
Cultured primary rat embryonic neurons from mesencephalon 6-OHDA (1.25-25 μM)	ASA (1 mM) ASA (1 mM)	Protection ??Mechanism Protection ??Mechanism
MPP^+ (0.625-20 μM) 141]. MPTP mouse model of PD [142].	Indomethacin (1 mg/kg, i.p.)	Protection ↓ inflammation
MPP^+ rat model of PD, intrastriatal 100nmol (in 4 μl/animal) [143].	SA (50 and 100 mg/kg, i.p.), diclofenac (5-100 mg/kg, i.p.) celecoxib (2.5-50 mg/kg, i.p.)	Protection ROS scavenging NO protection NO protection

Table 2. Continued

Experimental model	NSAIDs	Outcome
MPTP mouse (C57/BL/6) model of PD, MPTP (20 mg/kg i.p. four injections) 33].	Rofecoxib (12.5-50 mg/kg, i.p. for 5 days before and after	Protection ↓COX-2
MPTP mouse (C57/BL/6) model of PD, MPTP (20 mg/kg i.p) [144].	Rofecoxib	Protection ↓COX-2
MPTP mouse (C57/BL/6) model of PD, MPTP (60 mg/kg i.p) [41].	Rofecoxib (10 mg/kg, i.p., for 21 days 1 day after the injury MPTP)	NO protection
MPP^+ rat model of PD, intrastriatal (32 nmol in 1 μl) [146].	ASA (100 mg/kg, i.p. four injections, after MPP^+ infusion) Paracetamol (100mg/kg, i.p.four injections, after MPP^+ infusion)	Protection ROS scavenging Partial protection ROS scavenging
Rat mesencephalic neuronal cultures 6-OHDA (2.5-10 μM) MPP^+ (2.5-10 μM) [148].	Ibuprofen (25, 100, 250 μM) SC-560 (6.5 μM) NS-398 (5-50 μM) and Cayman 10404 (0.1-10 nM)	Protection for both toxins ↓COX-2 NO protection Protection against only 6-OHDA-toxicity ↓COX-2
6-OHDA rat model of PD, intrastriatal (22.5 μg) [147].	Celocoxib (20 mg/kg i.p., -1 up to +12 or 21 days)	Protection ↓COX-2
Primary mesencephalic mixed neuron-microglia cultures MPP^+ (0.5 μM) [23].	DuP697 (10 nM)	Protection ↓COX-2
Rat model of PD, intrastriatal MPP^+ or 6-OHDA 1 mM (10 min 1μl/min) [108].	ASA (100 mg/kg i.p.) Meloxicam (50 mg/kg i.p.)	Protection for both toxins ROS scavenging NO protection both toxins
Rat model of PD, intranigral 1 mM (32 nmol in 1 μl) [149].	ASA (100 mg/kg i.p.) Paracetamol (100 mg/kg i.p.)	Protection ROS scavenging and ↓superoxide anion generation
PC12 cells MPP^+ (30 μM) [150].	Indomethacin (100 μM) Ibuprofen (100 μM) Ketoprofen (100 μM) Diclofenac (100 μM) ASA (100 μM)	Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP No effect

ASA and SA but not for indomethacin was identified as COX-independent and in volved specific inhibition of GLU-mediated induction of NF-κB, suggesting, for the first time, a link between neuroprotection and the nuclear event [117].

Moreover, Aubin and colleagues [136] confirmed these ASA neuroprotective effects in a low dosage MPTP mouse model of PD. In accordance with the previous study, using an ex vivo and in vitro approach they found that the protective effect of ASA, its soluble lysine salt (Aspegic) and SA, is probably not due to COX inhibition. Their assertion was just a speculation based on the fact that other COX inhibitors such as paracetamol, diclofenac and indomethacin were ineffective. Likewise, the involvement of NF-κB was ruled out based on the lack of effect of dexamethasone, a glucocorticoid known to powerfully repress this nuclear factor function. ROS scavenging activity as a possible mechanism for explaining SA and ASA's neuroprotection was instead proposed by these authors [136].

In addition, SA was found to be neuroprotective, even if not completely, in a higher dosage MPTP mouse model of PD [137]. SA acted on both the terminal and cell body area of the nigrostriatal system as might be deduced by the pronounced effect against both MPTP-induced striatal DA depletion and loss of tyrosine hydroxylase (TH) immunereactive on nigral cell bodies. Ferger and colleagues in their paper pointed out that SA neuroprotective properties are based on its effective $^{\cdot}OH$ scavenger rather than on its COX-inhibitory action [137].

This piece of evidence has been further confirmed by Mohanakumar et al. [138], in a MPTP mouse model of PD, where SA demonstrated a clear antioxidant action blocking toxin-induced glutathione (GSH) and DA depletion acting as a $^{\cdot}OH$ scavanger in the brain and indicates its strength as a valuable neuroprotectant. SA did not inhibit MAO-B as has been previously shown by Aubin [136], overruling the possibility that its observed neuroprotective effects were caused by the possible blockade on the production of MPP^+ from MPTP due to the presence of this enzyme in the brain. It is worth noting that these authors showed for the first time that SA pretreatment also significantly improved motor activity, blocking akinesia or catalepsy caused by MPTP administration [138].

An in vitro study evaluated the effects of some NSAIDs on cultured primary rat embryonic neurons from rat embryos mesencephalon also containing glial cells, an experimental preparation that reflects the cellular composition of the brain well, and is therefore useful in the study of neuroinflammation [139]. Incubation with ASA, paracetamol or ibuprofen protected DAergic neurons against GLU toxicity, considering as indices the reduction of the decrease in DA uptake caused by GLU, and the attenuation of the TH-positive cells loss. Among the NSAIDs tested, ibuprofen was the most effective and surprisingly increased the number of DA cells in basal condition most likely protecting them from the excitoxicity associated with culture medium change [139].

So far experimental evidence has suggested that NSAIDs act as neuroprotectants essentially through a nonclassical mechanism. Against the general trend, the role of COX-1 and COX-2 enzymes was reassessed by Teismann and Ferger [140], who proposed the use of COX-2 inhibitors as a new non-DAergic therapy for PD. Their assumption was based on the effects of ASA and meloxicam, the latter a preferential antagonist for the COX-2 isoform, in a MPTP mouse model of PD. Both drugs, at higher dosages, showed an almost complete protection against MPTP toxicity. ASA and meloxicam antagonized MPTP-induced striatal DA depletion, attenuated the reduction of TH immunereactivity of the SNc and the MPTP-induced decrease in locomotor activity [140].

Carrasco and Werner [141], using a neuronally enriched mesencephalic culture system, showed that ASA was also able to increase the survival of DA neurons exposed to low doses of 6-OHDA and MPP^+ but not to counteract the morphological changes induced by the toxins. However, the authors did not investigate the possible protective mechanism of ASA [141].

The role of inflammation in the pathogenesis of PD was studied for the first time by Kurkowska-Jastrzębska et al. [142] using indomethacin in a MPTP mouse model of PD. This drug protected SNc DA neurons against the toxin effect and it was associated with diminished microglial activation and lymphocytic infiltration in the damaged areas. Thus, reduced inflammation by indomethacin might result in less damage of DAergic neurons. However, in this study, microglial and lymphocytes accumulation was decreased only in association with less neuronal impairment, when indomethacin was given before MPTP. Indomethacin in higher dose or given 24 h after intoxication did not decrease inflammatory reaction. Therefore, the anti-inflammatory effect of indomethacin might be secondary to the diminished neural injury which probably results from a direct interaction of indomethacin maybe on neurons scavenging ROS. However, indomethacin appeared to be toxic in high doses indicating that doses of NSAIDs should be considered carefully in clinical trials [142].

In view of conflicting reports so far on the role of the NSAIDs as neuroprotectants and the involvement of COX isoenzymes in their effects, Sairam et al. [143], used SA, diclofenac and celecoxib in a model of PD induced infusing MPP^+ directly into the striata of rats. These three anti-inflammatory agents have different mechanisms of action. SA is well-known to have an effect independent of the COX activity, diclofenac is a non-selective reversible COX-inhibitor and celecoxib is instead a specific COX-2 inhibitor. The failure of celecoxib and diclofenac to protect animals against MPP^+-induced DA depletion, together with a significant attenuation of severe DA depletion (>65%) induced by SA indicate the absence of the involvement

of prostaglandins (PGs) in MPP^+ action. The authors conclude that the difference in neuroprotection among the NSAIDs used in the study is mostly dependent on their antioxidant activity [143].

Further insights into the field have been provided by Teismann and colleagues [33] in a very elegant in vitro study from MPTP-treated mice and post-mortem PD samples. These researchers showed that COX-2 isoenzyme is up-regulated in the SNc DAergic neurons in both animal and human samples, COX-2-mediated neurodegeneration might be correlated to its catalytic activity through the production of prostaglandins and maybe also to the oxidation of catechols such as DA [112]. Treatment with rofecoxib, before and after MPTP-injection, blocked the increase of PGE_2 in the midbrain, doubled the number of the surviving TH-positive neurons, and prevented the rise in protein cysteinyldopamine, an index of DA quinones production. Surprisingly, neither pharmacological nor genetic abrogation of COX-2 activity mitigate inflammatory processes [112].

The neuroprotective effects of rofecoxib have also been shown by Klivenyi and colleagues [114] in MPTP model of PD in mice. They showed that the selective COX-2 inhibitor either alone or in combination with creatine, that facilitates metabolic channelling and shows antiapoptotic properties [145] protected against striatal DA depletions and loss of SN TH-immunoreactive neurons. Administration of rofecoxib with creatine produced significant additive neuroprotective effects against DA depletions. These results suggest that a combination of a COX-2 inhibitor with creatine might be a useful neuroprotective strategy for PD [144].

The work of Przybyłkowski and colleagues [41] is also noteworthy, they have shown, in the MPTP model of PD in mice, that rofecoxib has no neuroprotective effect when it is given after MPTP intoxication, even for a long period, revealing that the time of COX-2 inhibition is critical to achieve a protective effect. Consequently, COX-2 activity, prostaglandins production and oxygen species formation might not play a detrimental role in neuronal cells death, at least when the injury process has started already. Nonetheless, the inhibition of COX-2 activity could be harmful to neurons injured by MPTP. Indeed, the authors showed that, in later stages of injury, COX-2, through the formation of cyclopentenone prostaglandins derived from PGD_2, may participate in the resolution of inflammation and even in the regeneration process [41].

Otherwise, Maharaj et al. [146] showed that ASA given after MPP^+ administration, completely blockaded MPP^+-induced striatal DA depletion. Similar treatment with paracetamol resulted instead only in a partial protection. In both experimental conditions, rat brain homogenates and rats intranigrally treated with MPP^+, ASA and paracetamol acted mainly as antioxidants. They

were also capable of blocking $^{\cdot}OH$ production and lipid peroxidation in vitro, but in this regard ASA was the weaker compared to paracetamol. In conclusion, ASA appears to offer itself as a prophylactic as well as an adjuvant therapy for PD and its neuroprotective effect is only partially mediated by ROS scavenging properties [146].

Sánchez-Pernaute and colleagues [147] in a 6-OHDA rat model of PD, showed that selective inhibition of COX-2 by treatment (pre and post lesion) with celocoxib is protective against the neurotoxin effect. The authors evaluated celocoxib effects using micro PET and immunohistochemical techniques, and observed a decrease in microglial activation in the striatum and ventral midbrain associated with a prevention of the progressive degeneration seen in the intrastriatal 6-OHDA retrograde lesioned rats treated with the vehicle. The benefit of COX-2 activity inhibition might be attributed to a selective decrease of the harmful glial cells and to the no effect on the protective astroglia. Celocoxib's rescue of DA toxin-insulted neurons from death could be mediated by both neuronal and glial COX-2, but in any case the effect obtained by this drug is to create favourable conditions for the prevention of progressive neurodegenerative cascades during and after neuronal injury similar to that seen in PD [147].

On the other hand, results obtained in cultures of embryonic rat mesencephalic neurons treated with 6-OHDA and MPP^+ showed that these two neurotoxins act differently in the killing of DA neurons, neuronal COX-2 activity and PG production is involved only in the 6-OHDA-neurotoxic effect whereas MPP^+ toxicity does not require COX involvement [148]. This evidence comes from experiments carried out with ibuprofen, a non selective COX inhibitor, SC-560 a COX-1 selective inhibitor and two selective COX-2 inhibitors, NS-398 and Cayman 10404, showing that COX-2, but not COX-1, is involved in 6-OHDA toxicity. Since ibuprofen attenuated both 6-OHDA and MPP^+-neurotoxicity, the authors proposed that this drug has additional COX-independent effects as yet not well identified [148]. Some discrepancies with the previous study have been reported by Wang et al. [23]. These authors found that MPP^+ induces DAergic degeneration enhancing COX-2 expression in both glial and DA cells in primary mesencephalic mixed neuron-microglia cultures. Its toxicity is undoubtedly mediated through PGE_2, the levels of which almost doubled. They observed that the COX-2 specific inhibitor DuP697, attenuates microgliosis by decreasing PGE_2 production, and leads to the rescue DA neurons from a secondary lethal neurotoxicity attack [23]. Valdecoxib, an other selective COX-2 inhibitor, acted similarly in a mouse model of PD abating microglia activation and the consequential MPTP-induced toxicity, confirming that COX-2 and activated microglia play an important role in secondary injury of DA neurons. Moreover, these cellular protective effects of

valdecoxib pretreatment were confirmed in the behavioural counterpart of the experimentation in which it also alleviated locomotor deficits induced by the toxin, assessed in open field and vertical activity [23].

A recent study threw some light on this question by confirming that ASA has a protective effect against neuronal damage induced by intrastriatal infusion of MPP^+ and 6-OHDA using a microdialysis approach in conscious rats [108]. What makes this study noteworthy, is that the ASA neuroprotective effect was evidenced in vivo, indeed, this has been observed only under in vitro and ex vivo conditions to date. Pretreatment of rats with ASA, protected DA neurons in both animal models (MPP^+ and 6-OHDA-lesioned) as indicated by electrochemical and TH immunostaining evidence, whereas meloxicam, a selective COX-2 inhibitor, was devoid of any activity. The authors have confirmed these findings also in vitro, in a human neuroblastoma cell culture line. In fact, ASA, but not meloxicam, inhibited cell death induced by treatment with MPP^+, in a dose-dependent manner (unpublished observation). The mechanism of action of ASA seemed to be different in each model since it was associated with ROS scavenging activity in the 6-OHDA model, but not in the MPP^+ model that surprisingly did not induce any $^{\cdot}OH$ formation at the concentration used in this study. Therefore, it is likely that the protective effect exerted by ASA, in vivo, may be due to inhibition of MPP^+ toxicity at the cell level, possibly by blocking NF-kB or caspase activation, thus providing further evidence that the neuroprotective effect of NSAIDs might be independent from COX-2 inhibition. However, other mechanisms, such as $^{\cdot}OH$ scavenging activity, as in the model of 6-OHDA-induced damage, cannot be ruled out [108].

Finally, Maharaj et al. [149] have provided novel information by highlighting the role of NSAID agents on a different molecular target, the mitochondrion. These authors studied the effect of MPP^+ on striatal mitochondrial function and the ability of MPP^+ to generate superoxide $^{\cdot}OH$ and the effect of ASA and paracetamol. These NSAIDs prevented MPP^+-induced inhibition of the mitochondrial electron transport chain and complex I activity. In addition, ASA and paracetamol significantly attenuated MPP^+-induced superoxide anion generation. The results of this study suggest that these NSAIDs not only serve as $^{\cdot}OH$ scavengers but also prevent mitochondrial dysfunction and subsequent superoxide anion generation [149].

Nevertheless, Morioka et al. [150], have shown that treatment with some NSAIDs might instead aggravate the neurodegenerative processes. Indeed, coincubation of PC12 cells with indomethacin, ibuprofen, ketoprofen, or diclofenac, markedly enhanced MPP^+-induced cell death. This additive detrimental effect was not observed after treatment with ASA and NS-398, a

COX-2 selective inhibitor, that had no effects on the toxin action. The authors showed that the potentiating effect of some NSAIDs on MPP^+-induced cell death was not associated with any of the classical and non, actions attributed to them so far (i.e., inhibition of COX enzymes, ROS scavenging, antagonism at PPARγ, caspase-3-apoptotic cell death pathway). The possible mechanism whereby NSAIDs potentate MPP^+-induced cell death might be the increase of intracellular accumulation of MPP^+. In fact, these drugs suppressed the cellular efflux of MPP^+ by the blockade of multidrug resistance proteins (MRP) [150].

The use of different experimental conditions (i.e., in vivo versus in vitro, ex vivo, species or strain of animal used, cell types) drugs (NSAIDs are a heterogeneous chemical group also with different potency in crossing the blood-brain barrier), therapy duration (i.e., pre-treatment versus post-treatment or combination of both), time of observation, dose and type of neurotoxins used, may explain the differences among the studies here reviewed and, sometimes, the conflicting results. Overall, there is no doubt that ASA, SA, ibuprofen and especially COX-2 selective inhibitors exert neuroprotective effects, although the mechanism through which they act still remains controversial. Notwithstanding, the blockade of COX-2 activity seems to be essential for their effect. Their broad sites of action and pharmacological effects (from anticancer to antipyretic) might be the basis on which their efficacy in neurodegnerative disease is founded.

Neuroprotective effects of NSAIDs in Parkinson's diseases: Epidemiological evidence

Despite the evidence of inflammation in the brains of patients with PD, confirmed successively in animal models of PD, since the mid 1990s, NSAIDs have not yet been formally tested in PD. Hitherto, only five epidemiological studies have been carried out analyzing the association between regular use of NSAIDs and the risk of PD with conflicting results (Table 3).

Table 3. Epidemiological studies of NSAIDs and PD.

Study	Duration NSAIDs use	Overall cohort	PD cases	Relative risk	95% CI
Chen et al., 2003 [151]	>14 years	142,902	415	0.55	0.32-0.96
Chen et al., 2005 [153]	8 years	146,565	413	0.65	0.48-0.88
Case control	>3 years	7,896	1,258	0.93	0.80-1.08
Hernán et al., 2006 [154]		women	493	1.21	0.95-1.54
		men	765	0.79	0.65-0.96
Bower et al., 2006 [157]	20 years	404	202	0.50	0.20-1.5
Ton et al., 2006 [159]	20 years	589	206	0.90	0.59-1.35

The first piece of evidence was provided by Chen et al. [151] from the Harvard School of Public Health. They published the first study investigating prospectively the potential benefit in humans of the use of NSAIDs in reducing the risk of PD. These researchers found that regular users of these drugs had a lower risk of PD than non-users. The study was conducted among participants in the Health Professionals Follow-Up Study and the Brigham and Women's Hospital based Nurses Health Study who were free of PD, stroke or cancer at the start of the research. More than 44,000 men and nearly 99,000 women were followed for 14 years and 18 years, respectively. Use of ASA and non-ASA NSAIDs (such as, diflunisal, ibuprofen, indomethacin, naproxen) was assessed via biennial questionnaires. A total of 236 men and 179 women developed PD during the course of the study. The risk of developing PD was 45% lower among regular users of non-ASA NSAIDs compared to non-users (pooled multivariate relative risk (RR), 0.55; 95% confidence interval (CI), 0.32-0.96). Regular use of non-ASA NSAIDs was reported by 6.1% of the men at the beginning of the study and 3.7% of the women. A similar decrease in risk was also found among participants who took two or more tablets of ASA per day compared to non-users (RR, 0.56; 95% CI, 0.26-1.21). No benefit was found among those who took smaller amounts of ASA per day or paracetamol. Additionally, increasing benefits were observed with longer duration of use of non-ASA NSAIDs [151]. It is worth noting, that the Chen study may underestimate the protective effect of NSAIDs, since PD is much more common in people over 75 years old, an age group not included in the Chen team's data. Therefore, benefits of even greater magnitude might be demonstrable if this intervention were applied to the same population as it aged beyond 75 years. Moreover, these data provide little support for the routine use of NSAIDs as disease-modifying agents in PD, since to prevent one additional case of PD 98 individuals would have to be treated with them [152].

A subsequent prospective study conducted by the same group has provided further insights [153]. Chen and coinvestigators continued examining the relationship between NSAIDs use and risk of PD this time, utilizing another large cohort, the American Cancer Society's Cancer Prevention Study II Nutrition Cohort of 146,565 people. Between 1992 and 2000 they recorded 413 new cases of PD in the cohort. Ibuprofen was associated with 35% lower risk of PD (RR, 0.65; CI 95% 0.48-0.88), with similar risk reductions for men and women and regardless of age or smoking status. There was a significant trend for lower risk with increasing consumption of ibuprofen (from RR 0.73 with fewer than 2 tablets per week to RR 0.61 for daily use) but duration of use made little difference. In contrast to the previous study, no significant associations were found for ASA, other NSAIDs or paracetamol [153]. These discrepancies might be simply explained by the fact that considerably more people

in the cohort used ibuprofen than other medications. However, the authors also did not exclude that there may be an ibuprofen-specific effect against PD, related to its unique molecule. Furthermore, another limitation of this study is that because ibuprofen is an over-the-counter medication, the so-called "nonusers" could have taken ibuprofen years ago, therefore, a short-term clinical study might not give complete data. Instead, long-term data would be necessary to more fully discern users and nonusers.

Recently, another group from the Harvard School of Public Health, has conducted a case-control study on subjects with no history of PD or parkinsonism-related drug use at baseline [154]. Their study was nested within a cohort of the world's largest computerised database, the British General Practice Research Database (GPRD). The authors analyzed 1,258 PD cases and 6,638 controls, and reported a surprising finding: nonASA NSAIDs use reduces PD risk only in men but not in women. Use of nonASA NSAIDs was associated with a 20% reduction in the incidence of PD among men (odds ratio (OR), 0.79; CI 95% 0.65-0.96), and a 20% increase in the incidence of PD among women (OR, 1.21; CI 95% 0.95-1.54) [154]. Although sex differences in PD risk have been previously reported for caffeine consumption [155] and alcoholism [156], this was an unexpected finding that warrants further research.

Less promising insights have been provided by Bower and colleagues [157] from the Mayo Clinic College of Medicine in Rochester, Minnesota. They explored the association of PD with the use of NSAIDs in a population-based, case-control study for a total of 392 individuals. The investigators used the medical records linkage system of the Rochester Epidemiology Project to identify 196 subjects who developed PD from 1976 to 1995. Consistent with the previous epidemiological studies [151,153,154], Bower and colleagues found that cases of PD used NSAIDs (excluding ASA) less frequently than controls (OR 0.5; CI 95% 0.2-1.5); however, the difference did not reach significance. This trend finding was similar for both NSAIDs and steroidal agents considered separately. The use of ASA was not significantly associated with PD as shown previously [153,154]. These investigators also showed a significant association between pre-existing immune-mediated diseases and the later development of PD (OR 1.8; 95% CI: 1.1-3.1). The association was stronger for women and for earlier onset of PD cases, but neither of these differences reached significance. These results support the hypothesis that there is an inflammatory component in the pathogenesis of PD and provide a rationale for the use of NSAIDs as neuroprotectants capable of delaying onset or slowing progression of the disease [157]. Since patients with diseases of immediate-type hypersensitivity are genetically predisposed to initiate a humoral response to low levels of antigens, they might also be predisposed to

initiate neuroinflammatory responses as well and play a role in the aetiology of PD [158].

The latest available data on the subject come from Ton and colleagues [159] from the University of Washington and, unfortunately, they have continued in dampening the initial enthusiasm. In fact, in an American population-based case-control study these investigators did not observe a significant association between PD and NSAIDs. Subjects among enrolees of a health maintenance organization included 206 cases between ages 35 and 89 with a new diagnosis of idiopathic PD between 1992 and 2002, and 383 randomly selected controls. Exposure to NSAIDs was ascertained from an automated pharmacy database. After adjusting for age, sex, smoking, duration of enrolment, and clinic, the risk of PD among individuals who received non-ASA NSAIDs between 1977 and 1992 was 0.90 (95% CI: 0.59-1.35) and 1.67 (95% CI: 0.60-4.60) between 1993 and 2002. Use of ibuprofen was not associated with PD (OR: 0.89; 95% CI: 0.60-1.32). The risk of PD associated with ASA or ASA-containing medications was 0.74 (95% CI: 0.49-1.12). These results provide only limited support for the hypothesis that use of ASA may reduce the risk of this disease, but this association was statistically imprecise and no clear trend according to number of ASA prescription was observed. In addition, no indication at all of protection from other NSAIDs was revealed [159].

Differences in the methods of ascertaining medication exposures, in the extent or timing of exposure to NSAIDs, as well as chance, may account for the discrepant findings from this and the earlier studies. These findings offer, at most, a limited support for the hypothesis of neuroprotection from ASA, and no indication of protection from other NSAIDs. Larger studies that include medication records and over-the-counter medication use will clarify these associations. Nevertheless, these unclear indications must be clarified and corroborated by clinical trial before any firm conclusions can be drawn. Furthermore, the role of selective COX-2 inhibitors might be investigated since only the effect of traditional NSAIDs has been analysed by epidemiological studies. In fact, selective COX-2 inhibitors have not been in use long enough for epidemiological data to be collected. Moreover, the side effects of NSAIDs therapy, such as gastrointestinal lesions and cardiovascular risks should be carefully evaluated.

Conclusions and implications

From the large amount of literature here reviewed it appears evident that inflammatory processes are involved in the pathophysiology of PD. Neuroinflammation, a processes orchestrated and sustained by activated resident microglia cells, might be contributing to the demise of nigral DA cells, perpetuating the neurodegenerative phenomenon. A large body of information on the molecular and cellular mechanisms whereby inflammation might induce

neuronal death has been generated in the past few years by researchers in the neuroscience community. Nevertheless, further clarification of the role of inflammation in the pathophysiology of basal ganglia disorders is required, since the overall picture is still confusing. Complicating the situation is the fact that inflammation is a *double-edged sword* and probably starts as a beneficial defence mechanism that at some point evolves into a destructive and uncontrollable chronic reaction. Thus, the ideal approach would be to inhibit the deleterious effects associated with neuroinflammation while preserving the inflammatory pathways that lead to neuroprotection. From the above discussion it seems clear that drugs inhibiting inflammation and microglial activation might be an important feature of the treatment of PD and also the dementia, often associated with the disease [28,30,73,74,160]. Consequently, a rational use of NSAIDs could be useful as therapeutic intervention in PD and in other major neurological diseases with similar etiopathology, such as AD, ASL and MS. Nonetheless, despite the fact that experimental and epidemiological evidence has been provided for future use of anti-inflammation agents, they have not been rigorously corroborated in trial studies for the treatment of motor disorders as yet. Furthermore, most of the data have yielded contradictory results. This may be a result of the peculiar characteristics of these drugs, so different both at the chemical and action level. In fact, NSAIDs might exert their neuroprotective actions not only inhibiting COX enzymes but also by acting on NF-κB, iNOS, PPARγ, suppressing the formation of DA quinones, scavenging ROS and RNS activity and probably by other unknown mechanisms. Indeed, recently it has also been proposed that anti-inflammatory compounds might act inhibiting microglial proliferation, modulating the cell cycle progression and apoptosis [161].

NSAIDs are *sui generis*, and the further anti-inflammatory agents research progresses, the greater the number of indications that are discovered. NSAIDs have carved out a unique career in such diverse fields as the treatment of pain, migraine, prevention of cardiovascular disorders, and the chemoprophylaxis of various types of cancer. Probably, we are at the threshold of a new promising career for the NSAIDs especially in prevention of neurodegenerative disease rather than for their treatment. Indeed, it is quite possible that NSAIDs are ineffective once the pathological process has started, the pharmacological intervention should start very early in the pre-symptomatic period, according to some experimental a epidemiological evidence [41,151,153,154]. This need is also corroborated by the recent failure of some promising clinical trials in AD [162,163] shedding some doubts on the inflammation hypothesis of AD. Thus, the attractive thesis that NSAIDs might protect the remaining surviving DA neurons from the degeneration process and thus slow the ratio of progression of the illness sounds less promising. Due to the complexity of the disease, it is

possible that combination therapy, concomitant use of agents with nonoverlapping or even synergistic mechanisms of action, may represent the best means available to enhance treatment effectiveness. Some results could be achieved, therefore, by combining NSAIDs with other rescue agents, such as MAO inhibitors (rasagiline, safinamide); mitochondrial function enhancers (coenzyme Q10, creatine); antiapoptotic agents; protein aggregation inhibitors and neurotrophic factors [164]. Although this hypothesis is worthy of consideration, it remains largely undocumented and certainly deserves further discussion. Furthermore, NSAIDs might be a beneficial adjuvant to L-DOPA therapy counteracting the toxicity induced by its long-term use, through anti-inflammatory action and the reduction of DA quinones generated by L-DOPA therapy itself [112].

There are also many avenues that remain unexplored, so there are undoubtedly further advances to be made. In the next few years, we believe that novel approaches [164,165] will support the current dopamine-replacement therapy for PD. Furthermore, early diagnosis, early symptomatic treatment and particularly the introduction of neuroprotective therapies will improve PD pharmacological management. Indeed, disease modification remains the most important goal in PD. Consequently, compounds inhibiting neuroinflammation such as NSAIDs represent an important starting point that could lead us to the identification for the first time of disease-modifying agents for this devastating disease.

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